

What is the Precise Mechanism for Metal-independent Hydroxyl Radical Production by the Carcinogenic Pentachlorophenol Metabolites and Hydrogen Peroxide?

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Tetrachloro-1,4-benzoquinone (TCBQ) has been identified as a major genotoxic metabolite of the widely used wood preservative pentachlorophenol (PCP). PCP is now considered to be ubiquitously present in the environment and even found in people who are not occupationally exposed to it. PCP has been listed as a priority pollutant by the U.S. EPA, and classified as a group 2B environmental carcinogen by the International Association of Research on Cancer (IARC). Recently, we found that hydroxyl radicals ($\cdot\text{OH}$) are produced by TCBQ and hydrogen peroxide (H_2O_2) independent of transition metal ions. The production of $\cdot\text{OH}$ was measured by secondary radical ESR spin-trapping techniques, where $\cdot\text{OH}$ form methyl radicals upon reaction with dimethyl sulfoxide, and the methyl radicals are subsequently detected by ESR spectroscopy as the adduct with the spin trap 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO). In contrast, no $\cdot\text{OH}$ was detected from H_2O_2 and tetrachlorohydroquinone (TCHQ), the reduced form of TCBQ. However, if TCHQ was quickly oxidized to TCBQ by addition of myeloperoxidase, $\cdot\text{OH}$ could be detected. The antioxidants, ascorbate, dihydrolipoic acid and glutathione, completely inhibited the production of $\cdot\text{OH}$ by H_2O_2 and TCBQ. No correlation was found between $\cdot\text{OH}$ and tetrachlorosemiquinone anion radical (TCSQ^\cdot) formation. Thus, the production of $\cdot\text{OH}$ by TCBQ and H_2O_2 may not be through a previously proposed metal-independent organic Fenton reaction: $\text{TCSQ}^\cdot + \text{H}_2\text{O}_2 \rightarrow \text{TCBQ} + \cdot\text{OH} + \text{OH}^-$, in which TCSQ^\cdot substitutes for ferrous iron in the classic, metal-dependent Fenton reaction. Based on our recent results of reaction product analysis and other literature reports, a new mechanism is proposed: H_2O_2 may react with TCBQ or TCSQ^\cdot by a nucleophilic reaction, forming a phenylhydroperoxide intermediate. Because the oxygen-oxygen bond of phenylhydroperoxide is weak due to the stability of the resulting phenoxyl radical, $\cdot\text{OH}$ may be produced by homolytic fission of the hydroperoxide group: $\text{TCBQ} + \text{H}_2\text{O}_2 \rightarrow \text{TrCBQ-OOH} \rightarrow \text{TrCBQ-O}^\cdot + \cdot\text{OH}$ (TrCBQ-OOH: trichlorohydroperoxy-1,4-benzoquinone; TrCBQ-O $^\cdot$: trichlorohydroxy-1,4-benzoquinone radical). Further studies are needed to investigate this hypothesis. [Supported by NIH grants ES11497, 00210, RR01008 (BZ), HL60886 and AT00066 (BF)]